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Examiner: M. Meller **Group:** 1654

Date: October 16, 2003

Client Code: 2984

Facsimile No.: 703-308-0294

From: Elizabeth W. Mata, Esq.

Subject: **Papers:** **Remarks After Final Rejection, Amendment Fee Letter, and Exhibits A, B and C**

Docket No.: 2984.1000-004

Applicants: Yuan-Tsong Chen

Serial No.: 09/902,461

Filing Date: July 10, 2001

Number of pages including this cover sheet: 26

Please confirm receipt of facsimile: Yes _X_ No ___

Comments:

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DEB/EWM/cmm
10/15/03

PATENT APPLICATION
DOCKET NO. 2984.1000-004
Expedited Procedure under 37 C.F.R. 1.116
Examining Group 1654

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Yuan-Tsong Chen		
Application No.:	09/902,461	Group:	1654
Filed:	July 10, 2001	Examiner:	M. Meller
Confirmation No.:	6796		
For:	TREATMENT OF GLYCOGEN STORAGE DISEASE TYPE II		

CERTIFICATE OF MAILING OR TRANSMISSION

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10/16/03 — Date
Christina McSweeney — Signature
Christina McSweeney — Typed or printed name of person signing certificate

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith is a Remarks After Final Rejection for filing in the above-identified application.

[ ] Small entity status of this application under 37 C.F.R. 1.9 and 1.27 has been established by a Small Entity Statement previously submitted.

[ ] A Small Entity Statement to establish small entity status under 37 C.F.R. 1.9 and 1.27 is enclosed.

The fee has been calculated as shown below:

	(COL. 1) CLAIMS REMAINING AFTER AMENDMENT		(COL. 2) HIGHEST NO. PREVIOUSLY PAID FOR	(COL. 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDIT. FEE
TOTAL	22	MINUS	* 22	0	X $9	$ 0		X $18	$ 0
INDEP	3	MINUS	** 3	0	X $42	$ 0		X $84	$ 0
[ ] FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+ $140	$ 0		+ $280	$ 0

* not fewer than 20
** [illegible]

09/902,461

Please charge Deposit Account No. 08-0380 for the following fees:

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	TOTAL:	$ 0

[X] A general authorization is hereby granted to charge Deposit Account No. 08-0380 for any fees required under 37 C.F.R. 1.16 and 1.17 in order to maintain pendency of this application.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

David E. Brook, R.N. 22592

By for Elizabeth W. Mata
Elizabeth W. Mata
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Concord, Massachusetts 01742-9133
Dated: 10/16/03

@PFDesktop\::ODMA/MHODMA/iManage,423168,1
DER/EWM/cmm
09/26/03

PATENT APPLICATION
Attorney's Docket No.: 2984.1000-004
Expedited Procedure under 37 C.F.R. 1.116
Examining Group 1654

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Yuan-Tsong Chen		
Application No.:	09/902,461	Group:	1654
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Date Signature

Christina M. Sweeney
Typed or printed name of person signing certificate

REMARKS AFTER FINAL REJECTION

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

These remarks are submitted as requested by the Examiner at an interview on September 17, 2003 and in response to the Office Action, made final, mailed from the US PTO on June 3, 2003. A Notice of Appeal was mailed on October 3, 2003 and received at the US PTO on October 6, 2003.

REMARKS

Applicant's Attorney thanks the Examiner for granting the interview of September 17, 2003 and for his suggestions at the interview. Claims 1-9 and 11-23 are pending.

Rejections under 35 U.S.C. §112, second paragraph

The Examiner rejected Claims 1-9 and 11-23, stating that the term "periodically" was indefinite. This rejection is improper for the following reasons.

Words not specifically defined in the specification should be read as they would be by one of ordinary skill in the art. (M.P.E.P. 2111.01). Furthermore, a dictionary is an appropriate objective resource that can serve as a reliable source of information on established meaning that could be attributed to a claim term by one of skill in the art (see, e.g., Intellectual Property Development Inc. v. UA-Columbia Cablevision of Westchester Inc., 68 USPQ2d 1385 (CAFC 2003)). The Merriam-Webster Dictionary indicates that the term, "periodically," means "at regular intervals of time." For the Examiner's convenience, a copy of the Dictionary entry is attached as Exhibit A.

Further, the Specification provides several examples of what is intended by the term "periodically." See, e.g., p. 3, line 1, where it says "periodically (e.g., monthly, bimonthly, weekly, biweekly).. See also p. 9, line 21 where it says "periodically (as distinguished from a one-time dose)". This language supports the ordinary definition of the term, periodically, as it is set forth in the dictionary. Thus, one of ordinary skill in the art would understand that "periodically" refers to administration at intervals of time. This is further supported by the additional claim language regarding administration "at an administration interval."

Applicant's Attorney also notes that the term, "periodically" has been used in other patents relating to therapeutic application or administration of drugs or medications, without a specific definition of the term. See, for example, US Patents 4,735,802; 4,749,708; 4,761,417; 4,833,132; 5,292,754; 6,133,317; and 6,464,994.

In view of these considerations, the claims particularly point out and distinctly claim the invention, as one of ordinary skill in the art would understand the scope of the term, "periodically." Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims under 35 U.S.C. 102(b)

The Examiner rejected Claims 1-4, 9, 21 and 23 as being anticipated by Fuller *et al.*, stating that Fuller *et al.* describe treatment of Pompe's disease by administration of hGAA and that the enzyme is administered to an individual.

In order for a reference to anticipate a claim, each and every element at set forth in the claim must be found, either expressly or inherently described, in the reference (see, e.g., M.P.E.P. §2131). The following elements of the claims are neither expressly or inherently described by Fuller *et al.*: 1) administration *to a human individual*; 2) administration *periodically* and *at an administration interval*; 3) *treatment* of glycogen storage disease type II in a human individual.

Fuller *et al.* do not describe administration of GAA to anything other than cells in culture, nor do they describe administration of GAA periodically, at an administration interval. In addition, Fuller *et al.* do not describe "treatment" of disease in an individual, as that term is described in the current Specification and would be understood by one of ordinary skill in the art. Neither uptake of enzyme by cultured human fibroblasts, nor uptake of enzyme by cultured human skeletal muscle cells and subsequent processing of lysosomal glycogen in the muscle cells, both occurring in the short term (e.g., 12 to 24 hours) indicates whether administration of the GAA to a patient periodically, at an administration interval, will treat the disease (e.g., by ameliorating one or more symptoms associated with the disease, preventing or delaying the onset of one or more symptoms of the disease, and/or lessening the severity or frequency of one or more symptoms of the disease). It is known in the art that *in vitro* cell culture conditions differ significantly from *in vivo* conditions: for example, when enzyme is administered *in vivo* by intravenous infusion, the muscle cells don't come into direct contact with enzyme as they do in cell culture. Furthermore, the endothelial barrier as well as the interstitial connective tissue must be passed *in vivo*. See, for example, Reuser *et al.* (Eur. J. Pediatr. 161:S106-S111 (2002); a copy of which is attached for the Examiner's convenience as Exhibit C).

Furthermore, Fuller *et al.* cannot describe treatment of cardiomyopathy, as set forth in Claim 21, as they do not describe administration of enzyme to heart cells. In view of these considerations, Fuller *et al.* do not teach each and every aspect of the claimed invention. Therefore, the claims are not anticipated by the teachings of Fuller *et al.*

Applicant's Attorney notes that the Examiner stated that Claim 21 only requires that the "human individual" has glycogen storage disease type II. If the Examiner believes that amendment of the claim to specify that the human individual has cardiomyopathy, Applicant would be willing to consider doing so.

Rejection of Claims under 35 U.S.C. §103

The Examiner rejected Claims 1-7, 11-18, 21 and 23 as anticipated by, or in the alternative, as obvious over, Fuller *et al.* The Examiner also rejected the claims as being obvious over Fuller *et al.*

As indicated above, Fuller *et al.* state that they believe that the precursor GAA "will be a useful candidate for replacement therapy in GSD II patients." Assuming *arguendo* that one of ordinary skill in the art was motivated to try to treat Pompe's disease by this teaching of Fuller *et al.*, the current invention would nevertheless not have been obvious, because one of ordinary skill in the art would not have had a reasonable expectation that treatment would be successful.

The terrible effects of Pompe's disease and Dr. Chen's successful treatment are described by the Muscular Dystrophy Association (MDA) in the publication QUEST (Volume 10, Number 2, March/April 2003). A copy of this publication was left as a courtesy with the Examiner during the interview on September 17. For convenience, a copy is also attached as Exhibit B.

Pompe's disease is a rare disease which is extremely devastating for the afflicted individuals; infants with the disease are not expected to live beyond one year of age. The disease has been known for about seventy years, since the early 1930's, and the enzyme deficiency has been known for about forty years, since the 1960's. And yet, many attempts at treatment of the disease by administration of replacement enzyme have failed. See, for example, Van der Ploeg *et al.* (J. Clin. Invest 87:513-518 (1991), cited in IDS as reference AW3, which lists several references that indicate that attempts at enzyme replacement therapy have failed); Williams *et al.* (Birth Defects: Original Article series Volume XVI, no. 1, pp. 415-423 (1980), cited in IDS as reference AW, which states that a preliminary trial to treat a terminally ill patient with Pompe disease was not clinically successful); and de Barsy *et al.* (Birth Defects: Original Article Series, Vol IX. No. 2, pp. 184-190(1973), cited in IDS as reference AU2, which states that no conspicuous morphologic or clinical improvements were noted after an attempt to treat a patient with enzyme; that no morphologic or biochemical evidence of replacement therapy had been obtained to date; and that it appeared that the enzyme was not being transported to the relevant places in the body).

Dr. Chen has demonstrated successful treatment of this genetic disease by administration of the enzyme to a human individual. As described in detail in the Example in the application,

periodic administration of GAA produced in Chinese hamster ovary cells to three separate patients, resulted in significant amelioration of symptoms associated with the disease, as well as delay in onset of more severe symptoms. For example, significant improvements in cardiac parameters were noted; pulmonary function and skeletal muscle functions improved and remained normal in one patient; neurologic and developmental characteristics were improved or remained normal. The successful reversal of certain symptoms in all patients, as well as the normal muscle functions, neurologic and developmental characteristics of the third patient, were highly significant because it was previously unknown whether human symptoms could be alleviated or whether normal development could be achieved by administration of GAA. Without treatment, these children were expected to die; as reported in the attached MDA article, most infants with the disease aren't expected to live to one year of age. Dr. Chen's invention, on the other hand, is a successful treatment of an otherwise fatal genetic disease affecting heart and muscle. The unexpected nature of this success is further emphasized in the Declaration under 37 C.F.R. §1.132 of Dr. Chen (the "Declaration"), previously submitted.

In view of the long-felt need for a treatment, the failure of others to treat Pompe's disease successfully, and the unexpected success achieved by the inventor, the claimed invention would not have been obvious over the teachings of Fuller *et al.*

The Examiner rejected Claims 1-9 and 11-23 under 35 U.S.C. 103(a) as being unpatentable over Bijvoet *et al.* in view of Fuller *et al.*, stating that it would have been obvious to use the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*

Bijvoet *et al.* do not describe administration of GAA to a human individual, as is required by the claims. In addition, Bijvoet *et al.* do not teach or suggest administration of GAA periodically, at an administration interval, nor do they describe "treatment" of disease in a human individual.

One of ordinary skill in the art, given the teachings of Bijvoet *et al.*, would not have been motivated to look to the teachings of Fuller *et al.* regarding enzyme produced in CHO cell culture; in fact, Bijvoet *et al.* teach away from use of enzyme produced in CHO cells: they indicate that high production costs associated with use of enzyme produced in CHO cells are a

significant concern and discuss experiments designed to provide proof of principle for obtaining enzyme by other means (Bijvoet *et al.*, p. 1820, "Discussion").

Even assuming *arguendo* that the teachings of Bijvoet *et al.* were combined with the teachings of Fuller *et al.*, one of ordinary skill in the art would not have obtained the present invention. One of ordinary skill in the art, using the enzyme of Fuller *et al.* in the methods of Bijvoet *et al.*, would have been motivated only to administer a single dose of the enzyme, and not to administer enzyme periodically at an administration interval. Furthermore, given the teachings of Bijvoet *et al.* in combination with the teachings of Fuller *et al.*, one would not have had a reasonable expectation of successfully treating the human individual. As discussed above, there was a long-felt need for a treatment and significant failure by others in the art to achieve treatment by administration of enzyme. One of ordinary skill in the art would not have known, given the teachings of Bijvoet *et al.* regarding uptake of enzyme by cultured human fibroblasts, and increase of enzyme activity in knockout mice administered a single dose of the enzyme, whether periodic administration, at an administration interval, to a human individual, of GAA as taught by Fuller *et al.*, would in fact successfully "treat" the patients. In view of these considerations, the claimed invention would not have been legally obvious under Section 103 over the teachings of Bijvoet *et al.* in combination with Fuller *et al.*

SUMMARY

The claims particularly point out Applicant's invention, specifying that a human individual is treated with GAA periodically, at an administration interval. The Fuller *et al.* reference used in the rejections under 35 U.S.C. 102 fails to teach every aspect of the claimed invention, as it does not teach treatment of a human individual by administration of hGAA periodically at an administration interval.. Furthermore, the cited references (Bijvoet *et al.* and Fuller *et al.*), either alone or in combination, do not teach or suggest treatment of a human individual having GSD-II, by periodic administration of hGAA at administration interval, wherein the hGAA is produced in CHO cell culture. Furthermore, given the teachings of the references, one of ordinary skill in the art would not have had a reasonable expectation that disease could, in fact, be successfully "treated" by administration of the hGAA periodically at an

administration interval. Thus, the claimed invention would not have been obvious over the cited references. Dr. Chen has demonstrated successful treatment of this terrible genetic disease after all of the failures of others.

CONCLUSION

In view of these considerations, the claims are in condition for allowance. Applicant's Attorney requests that the Examiner reconsider and withdraw all objections and rejections.

If the Examiner believes that a telephone conversation would expedite prosecution of the application, the Examiner is invited to call Elizabeth W. Mata at (915) 845-3558. If Elizabeth W. Mata cannot be reached, the Examiner is invited to call David E. Brook at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

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By for Elizabeth W. Mata

Elizabeth W. Mata
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Concord, MA 01742-9133

Dated: 10/16/03

EXHIBIT A

Main Entry: pe·ri·od·i·cal·ly
Pronunciation: "pir-E-'ä-di-k(&-)lE
Function: adverb
Date: 1646
1 : at regular intervals of time
2 : from time to time : FREQUENTLY

1. Pronunciation Key

EXHIBIT B

QUEST Volume 10, Number 2, MARCH/APRIL 2003

Pompe's Disease

A Killer Yields to Modern Medicine

by *Dan Stimson*

You may never have heard of Pompe's disease. It affects just 5,000 to 10,000 people in the United States, making it exceedingly rare and of little interest to the general public. But what it lacks in notoriety, it makes up for in personal devastation to those who have it.

Robert Elmore nuzzles his son Grante ("Nikko"), who has lived twice as long as expected thanks to enzyme replacement therapy provided in a clinical trial.
Photos by Amy Snyder

Pompe's (also known as acid maltase deficiency) is caused by a genetic deficiency of an enzyme that breaks down glycogen (stored sugar) inside muscle cells. In its severest form, it strikes during infancy, weakening the heart and the voluntary muscles, including those that control breathing. The disease can also manifest during childhood or adulthood, causing significant muscle weakness and respiratory problems.

Children and adults with the disease usually have a shortened life span, and most infants with the disease aren't expected to live beyond 1 year of age.

But these grim prognoses could soon change, thanks to research led by Yuan-Tsong Chen, professor and chief of Medical Genetics in the Department of Pediatrics at Duke University in Durham, N.C., and director of the Institute of Biomedical Sciences, Academia Sinica, Taiwan. Through basic research supported by MDA and clinical trials supported by the biotech company Genzyme, Chen and his team at Duke have developed a way to supply the missing enzyme to people with Pompe's disease.

In two trials, one completed in 2000 and the other last year, 11 babies have received this experimental treatment — called enzyme replacement therapy — and some are now healthy, walking toddlers.

A Faulty Enzyme, Failing Muscles

With currently available treatment, "There's not much we can do for babies with Pompe's disease," Chen says.

Within weeks or months of birth, an infant with the disease can become too weak to suckle or breathe on its own. The muscular walls of the heart become enlarged, shrinking the heart's inner chambers and reducing its pumping capacity, a condition known as *hypertrophic cardiomyopathy*. Most babies with the disease die from cardiac and respiratory failure within three to four months of diagnosis, Chen says.

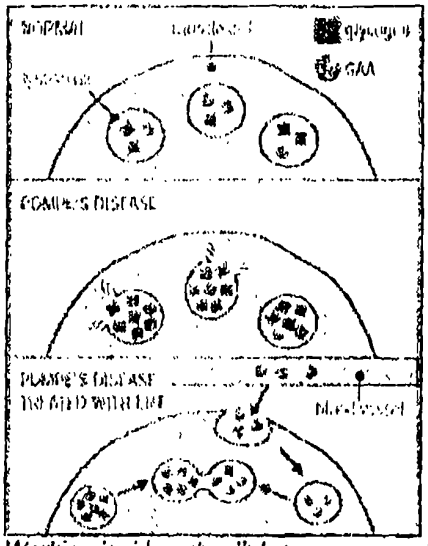
This picture of infant-onset Pompe's disease has changed little since it was first described by Dutch pathologist Joannes Pompe in the early 1930s. While studying at the University of Amsterdam, Pompe was asked to do an autopsy on a 7-month-old girl who had been admitted to the university hospital with difficulty breathing and had died three days later, apparently of pneumonia. Expecting to see her lungs filled with fluid, he was surprised to find that her heart had swollen to more than three times its normal size and the cells within it were filled with clumps of debris, which turned out to be glycogen.

It wasn't until the 1960s that other researchers discovered the underlying basis of Pompe's disease — a deficiency of the enzyme *acid alpha-glucosidase (GAA)*, also called *acid maltase*. The deficiency can now be detected by blood tests that probe for GAA activity or for mutations in the gene encoding GAA, located on chromosome 17. It's estimated that one in 85 to 100 people carries a mutation in a single copy of the gene; it takes mutations in both copies, one inherited from each parent, to cause the disease.

The severity of mutations in the GAA gene — that is, how much they alter the enzyme — determines, at least in part, the severity of the disease.

Mutations that destroy the protein cause infant-onset Pompe's disease, while mutations that leave some GAA intact tend to cause juvenile- and adult-onset forms of the disease.

The later-onset forms of Pompe's are primarily "muscle diseases," Chen says. Cardio-myopathy is mild in the juvenile form, and usually absent from the adult form. In children, the most common first symptom is delayed motor development; in adults, it's difficulty walking. For both late-onset forms, respiratory weakness can be severe and often requires mechanical ventilation, Chen says.



Working inside subcellular compartments called lysosomes, the enzyme acid alpha-glucosidase (GAA) breaks down glycogen (top). In Pompe's disease, a deficiency of GAA causes glycogen to accumulate and rupture lysosomes (middle). In enzyme replacement therapy (ERT), intravenously injected GAA is taken up by lysosomes that have fused with the cell's outer surface, eventually making its way to glycogen-filled lysosomes (bottom).

The Lysosome Connection

Chen has been studying glycogen storage disease, a category of diseases that includes Pompe's, for more than 20 years. (Pompe's disease is sometimes called *glycogen storage disease type 2* or *acid maltase deficiency*; it's one of 10 *metabolic diseases of muscle* covered by MDA's program.)

Early in his career, he recognized that Pompe's disease was going to be a tough nut to crack.

In most glycogen storage diseases, inadequate breakdown of glycogen leads to hypoglycemia, a drop in blood sugar levels that drains the body of energy. In these diseases, supplementing the diet with complex sugars like cornstarch can help maintain blood sugar levels and control symptoms.

But the symptoms of Pompe's disease aren't related to hypoglycemia; instead, they're caused by the accumulation of glycogen itself. GAA is one of many enzymes found in *lysosomes*, compartments inside cells that "clean house" by trapping and degrading glycogen and other chemicals. Without GAA, glycogen builds up inside lysosomes and ruptures them, an effect that's especially damaging to muscle, which naturally makes large amounts of the energy-rich substance. (This makes Pompe's disease a glycogen storage disease *and* a lysosomal storage disease, a category that includes Tay-Sachs and Niemann-Pick diseases.)

Chen has thus focused much of his research on how to deliver GAA to the lysosomes of people with Pompe's disease. This is a tall order, one that might seem to parallel efforts at gene therapy and stem cell therapy for

muscle disease, which haven't yet shown success in the clinic.

But Chen has been able to exploit a key feature of lysosomes: In their business of "housecleaning," they fuse with the outer surface of the cell, allowing them to release their contents and take up substances from the outside. Because lysosomes take in other substances, Chen and others reasoned that intravenously delivered GAA might make its way into the lysosomes of muscle cells.

Yuan-Tsong Chen.
Photos by Cramer Gallimore

Building a Better Enzyme

Enzyme replacement therapy for Pompe's disease wasn't his idea, Chen acknowledges. In clinical trials in the 1970s, patients with the disease were given injections of GAA isolated from human placenta, but the treatment failed.

Later, Chen says, "We learned that in order for the enzyme to work, you need to make a special form of it that can be taken up by the cells in [voluntary] muscles and in the heart. The second critical issue is how to make sufficient quantities of the enzyme for a clinical trial."

By the 1980s, scientists discovered that for efficient uptake by lysosomes in muscle cells, GAA and other enzymes must have a chemical "tag," called *mannose-6-phosphate (M6P)*. Human placenta had been a plentiful source of GAA, but it produces a version of the enzyme that has very little M6P.

In the 1990s, with advances in molecular biology and funds from MDA, Chen was able to engineer an M6P-laden version of the enzyme — called *recombinant human GAA (rhGAA)* — using a line of cells (*CHO cells*) to produce it in large amounts.

Armed with this new enzyme, Chen formed a collaboration with researchers from Tokyo, who were studying a strain of Japanese quail rendered flightless by naturally occurring Pompe's disease. After three weeks of injections with rhGAA, the birds could fly.

Chen and his team were ready to test rhGAA in babies with Pompe's disease, but first, they needed help from the biotech industry.

"In order to test the therapy in humans," Chen explains, "we needed to make the enzyme in a GMP [good manufacturing


Andy Amalfitano

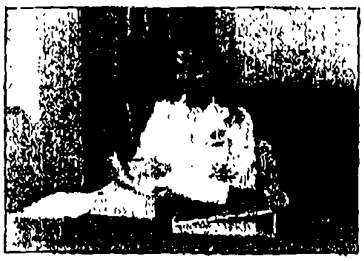
practice] facility, we needed to have every single step documented, and we needed large bioreactors" — incubation chambers for growing the CHO cells that produce rhGAA. "These are things we're not able to do in an academic research lab."

A Lifesaving Treatment

Working first with Synpac, a pharmaceutical company based in Taiwan, and later with Genzyme, a Cambridge, Mass.-based company with a longstanding interest in lysosomal storage diseases, Chen began his first trial of enzyme replacement therapy for Pompe's disease in 1999. The results were published in March 2001 in the journal Genetics in Medicine.

The three babies in the trial, who ranged from 2 months to 4 months old at its inception, had once been expected to die — but all of them are still alive. After about a year of twice-weekly intravenous infusions with rhGAA, all experienced significant reductions in heart size and improvements in cardiac function. Genzyme has continued to supply them with the treatment.

One baby has become "an essentially normal 3-year-old boy," able to walk and to breathe on his own, Chen says. The other two, now 3 and 4 years old, require mechanical ventilation and haven't developed normal motor skills, but they have normal cardiac function, he says.


Priya Kishnani

In 2001, Genzyme and Duke scientists launched a second trial involving eight babies, ranging from 3 months to 14 months old. Five of the infants were studied at Duke and the others were studied at sites in Europe. Details of the results await publication, but Priya Kishnani, the trial's lead investigator at Duke, presented some of her data at a scientific meeting in Dublin in September.

According to her report, all of the babies experienced significant reductions in heart size, two died from complications unrelated to the enzyme, and the remaining six were still alive after about a year of treatment. (For more about one of these toddlers, see "A Time to Celebrate.")

"It's such a fruitful experience to go from a diagnostic approach to a treatment approach for a disease that's considered lethal," Kishnani says. "By no means is this a cure; we don't know the long-term benefits or side effects of the treatment. But there's nothing else out there right now to change the natural course of this devastating disease."

What the Future Holds

This year, Genzyme and the Duke team, led by Kishnani, will begin two larger trials of enzyme replacement therapy for Pompe's disease.

The trials are a final step toward getting the treatment approved by the U.S. Food and Drug Administration, Chen says. One, already under way, is enrolling toddlers between 6 months and 3 years old and the other will enroll babies less than 6 months old. (For more information, contact Genzyme Medical Information at [800] 745-4447.) Each trial will recruit up to 16 patients and will test a different version of rhGAA than that used in the previous trials.

Genzyme, which has made Pompe's disease its largest research and development effort since its founding 21 years ago, now has an arsenal of rhGAA types. The company began testing enzyme replacement therapy for Pompe's disease in 1998, through a joint venture with Pharming, a Dutch biotech company. Scientists from the two companies genetically engineered rabbits to produce rhGAA in their milk, and had begun testing this "transgenic" rhGAA in patients with the infantile and juvenile forms of Pompe's disease. In 2001, Pharming went into receivership and Genzyme acquired the rights to the transgenic rhGAA.

Genzyme acquired another type of rhGAA, made in CHO cells like Chen's, when it bought the Princeton, N.J., company Novazyme Pharmaceuticals.

Recently, Genzyme has developed a fourth version of rhGAA with "improved scalability," meaning it can be produced in larger quantities than previous versions. This is the enzyme that Duke researchers will test in upcoming trials; once a sufficient amount of the enzyme is available, they hope to test it in adults with Pompe's disease.

Looking to the more distant future, scientists at Genzyme and Duke are also investigating gene therapy for Pompe's disease. One potential benefit of this approach is "a decreased need for frequent infusions of the enzyme. You could envision a gene therapy treatment that would only be required yearly," says Andy Amalfitano, a co-investigator in the enzyme replacement therapy trials.

In fact, Amalfitano says, "Pompe's disease may be one of the best diseases to consider treating by gene therapy... because we have an opportunity to treat every muscle in the body without inserting the [GAA] gene into every muscle." A virus could be used to deliver GAA to the liver, which could then release the enzyme into the bloodstream, Amalfi-tano explains. In MDA-funded experiments at Duke, he's used this approach to restore GAA activity to the muscles of mice and quail with Pompe's disease.

Editor's Note: Until now, MDA and Genzyme have made independent efforts to support the development of enzyme replacement therapy for Pompe's disease. In November, MDA and Genzyme staff met at MDA's National Headquarters in Tucson, Ariz., and discussed plans to collaborate on future research.

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EXHIBIT C

Eur J Pediatr (2002) 161: S106–S111
DOI 10.1007/s00431-002-1015-8

ORIGINAL PAPER

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Enzyme therapy for Pompe disease: from science to industrial enterprise

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Abstract Pompe disease or glycogen storage disease type II (OMIM 232300) is a metabolic myopathy with a broad clinical spectrum. Generalised muscle weakness combined with cardiomegaly presents within the first 3 months after birth, if the lysosomal α-glucosidase (AGLU) deficiency is complete. Residual enzyme activity prevents cardiac involvement and delays onset of muscle weakness. Enzyme therapy, by intravenous administration of acid AGLU, aims to supplement the missing enzyme activity. At the SHS symposium on Glycogen Storage Diseases Type I and II, in Fulda, two interim accounts were given of studies on the efficacy of enzyme therapy for Pompe disease; one with recombinant human acid AGLU produced in Chinese hamster ovary cells and the other with the same enzyme produced in the milk of transgenic rabbits. *Conclusion:* this review focuses on the latter study, discusses the scientific, technological and commercial aspects of the enterprise, and addresses the prospects and challenges of enzyme therapy for Pompe disease.

Keywords Acid maltase deficiency · Enzyme therapy · Glycogenosis · Lysosomal storage disease · Transgene technology

Abbreviations *AGLU* α-glucosidase · *CHO* Chinese hamster ovary · *CRIM* cross-reactive immunological material · *M6P* mannose-6-phosphate

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Enzyme therapy in historic perspective

Over the past 35 years we have learned what it takes to bring enzyme therapy for lysosomal storage disorders into practice. The concept of enzyme therapy is built on the key function of lysosomes in cell and tissue renewal. Macromolecular compounds, even whole cell organelles like mitochondria, are recycled by the lysosomal system (Fig. 1). Materials derived from the intra-cellular space are sequestered by membranes and delivered through fusion of newly formed autophagic vacuoles with lysosomes. Extra-cellular materials are taken up by bulk or receptor mediated endocytosis and are delivered by fusion of endosomes with lysosomes. Once inside lysosomes, the material is degraded by one or a combination of several lysosomal hydrolases. Some of these hydrolases are assisted by activator proteins [23].

The role of lysosomes in cellular pathology became evident in 1963 with the discovery of acid α-glucosidase (AGLU) deficiency as primary defect in Pompe disease or glycogen storage disease type II [13]. Knowing the function of lysosomes, it was envisaged that patients with lysosomal storage disorders could be treated by administration of the missing enzyme that would find its way to the lysosomes via endocytosis (Fig. 1) [9]. Expectations were high and regulations concerning the performance of clinical studies were less strict in those days than they are at present. The first attempt at enzyme therapy dates from 1964 and involved treatment of a patient with Pompe disease with acid AGLU from the fungus *Aspergillus niger* [5]. Similar experiments followed using enzyme preparations from various other sources [30]. A slight increase of acid AGLU activity in liver was obtained in some of these studies upon intravenous infusion [19], but not in muscle. Reduction of liver lysosomal glycogen only was obtained with high enzyme doses over long periods of time. In all instances, lack of ultimate effect and occurrence of serious side-effects terminated the treatment.

In the period 1965–1980, numerous reports were published about enzyme replacement therapy in several of the lysosomal storage diseases, but most results were basically negative [30]. Some important facts became evident. Enzyme preparations from non-human sources are antigenic. The blood brain barrier cannot be crossed, so that patients with central nervous system involvement cannot be treated for mental and motor dysfunction.

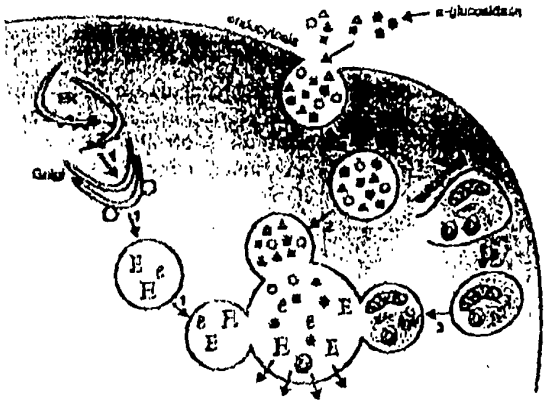

Fig. 1. Enzyme replacement therapy in lysosomal storage diseases. Newly synthesised lysosomal enzymes enter the lumen of the endoplasmic reticulum co-translationally. They are glycosylated, folded, and equipped with the M6P recognition marker as lysosomal targeting signal (route 1). Inside lysosomes, they catalyse the digestion of biological compounds that are delivered by endocytosis (extracellular) (route 2) and autophagy (intracellular) (route 3). Inherited deficiency of lysosomal enzymes leads to lysosomal storage diseases. Lysosomal enzymes administered to cells are taken up via bulk (inefficient) and receptor mediated (efficient) endocytosis and are delivered to the lysosomes where they can supplement the missing enzyme. (*E* enzymes, *ER* endoplasmic reticulum)

Advanced technology is required to secure substantial enzyme supplies over long periods. Around 1980, the focus shifted from enzyme therapy to bone marrow transplantation [15].

A few people pursued the original concept and gave enzyme therapy a second and better chance by applying newly acquired knowledge on receptor mediated endocytosis. Ashwell and Morell [2] were among the pioneers to demonstrate the role of the asialoglycoprotein receptor (galactose) in uptake of glycoproteins by hepatocytes. The mannose receptor was shown to facilitate uptake of proteins with mannose-terminating carbohydrate side chains by Kupffer cells and macrophages of spleen and bone marrow [29], and fibroblasts were found to exchange lysosomal proteins via the mannose 6-phosphate (M6P) receptor [14, 21]. Brady and his group [3] were the first to demonstrate the potential of receptor mediated enzyme therapy for lysosomal storage disease in a patient with Gaucher disease. The glucocerebrosidase used in the study was purified from human placental tissue. The complex carbohydrate side chains were trimmed by sequential action of neuraminidase, galactosidase and N-acetyl-glucosaminidase in order to expose mannose residues and thereby target the enzyme to Kupffer cells and macrophages in spleen and bone marrow; the major sites of lysosomal glycolipid storage in Gaucher disease. The study turned out successful enough to convince scientists, patients and industry of the feasibility of receptor mediated enzyme therapy for Gaucher disease [4].

Enzyme therapy for Pompe disease

We have worked along parallel lines to investigate the feasibility of receptor mediated enzyme therapy for Pompe disease. Table I lists the critical events by date.

Table I. Enzyme therapy for Pompe disease from science to industrial enterprise

Event	Production	
	Milk enzyme	CHO enzyme
Enzyme therapy: proof of principle	[35]	
AGLU cDNA cloning	[16]	
	[24]	
AGLU gene cloning	[17]	
	[25]	
Production	[6]	[11]
	[7]	[36]
Testing in animals	[7]	[36]
	[8]	[22]
		[7]
Industry enters the scene	Pharming (1991)	Synpac (1994)
	Genzyme (1998)	KDL BioTech (2000)
		Genzyme (2000)
		Pharming (2000)
		Novazyme (2000)
Orphan drug designation	1996	1997
Phase I trial	1998	-
Phase II trial	1999	-
Phase I/II trial	-	1999
Phase II trial data	[31]	[1]
Phase II/III trial	Not planned	May 2001

The target tissues in Pompe disease are muscle and heart. Patients have the same acid AGLU deficiency in all tissues, but the lysosomal glycogen accumulation and the symptomatology are largely restricted to skeletal muscle when the residual enzyme activity is 5%-25% of the normal range. At lower activity levels, heart and other tissues become increasingly involved. There is typically a clinical spectrum from early onset very severe to late onset mild disease. Affected infants have cardiomegaly around birth. They present as floppy babies and die usually before 1 year of age due to cardiorespiratory failure. Onset of symptoms is delayed, and cardiomegaly prevented by low levels of residual AGLU activity. In extreme cases, the skeletal muscle weakness may remain obscure until the sixth decade [27].

Our initial studies on the feasibility of enzyme replacement therapy for Pompe disease were directed towards establishing the presence of M6P receptors on cardiomyocytes and skeletal muscle cells and testing whether these could be employed to facilitate uptake of acid AGLU [26]. To this end, muscle biopsies of patients were dissociated with collagenase and trypsin, and myoblasts (satellite cells) were taken into culture. It turned out no problem to correct the lysosomal glycogen storage in these cells by addition of M6P-containing acid AGLU to the culture medium [32, 33, 34]. Herewith, the first requirements were fulfilled, but the experimental set-up does not mimic the reality in detail. When enzyme therapy is performed via intravenous infusion, the muscle cells do not come into direct contact with the enzyme like they do in tissue culture. The endothelial barrier of the capillaries needs to be crossed and the interstitial connective tissue (endomysium) needs to be passed. In this respect, the situation in Pompe disease is far different from that in Gaucher disease where there are no barriers between the Kupffer cells and the enzyme in the circulating blood. In Fabry disease the situation also is favourable compared to Pompe disease because the endothelial cells are a primary target. Nevertheless, we did obtain uptake of acid AGLU in muscle and heart of mice after intravenous administration of M6P-containing AGLU from bovine testis. The uptake of AGLU without M6P was less [35].

Technical challenges and financing

It took another 8 years before the first clinical trial of enzyme therapy in Pompe disease started. The time was spent on the development of technology to produce therapeutic grade recombinant human AGLU on a large scale. Therapy for Gaucher disease started with the heroic action of Genzyme Corporation (Boston, USA) to produce tailor-made glucocerebrosidase from tons of human placentas. The investment paid off for all parties. Patients with non-neurological forms of Gaucher disease (type 1) obtained an effective medicine [12]. Scientists were pleased because their ideas were realised. The company had shown its strength by bringing a new drug on the market and obtained returns.

The Orphan Drug Legislation, lending certain attractive rights to companies marketing medicines for rare diseases, has played an important role in this development. The past 5 years have shown concerted action of scientists, patients, industry and investors to be a golden formula for developing enzyme therapy for lysosomal storage diseases. Belief in enzyme therapy has returned. More importantly, the first reports confirming efficacy have appeared and clinical trials are ongoing for at least four of the lysosomal storage diseases [1, 10, 20, 28, 31]. In all instances, the protocols are directed to receptor-mediated tissue targeting, and recombinant DNA technology is applied for controlled large-scale enzyme production. The investment climate is excellent. The market seems profitable enough to have several companies competing for the same product. Both TKT (Boston, USA) and Genzyme Corporation have launched enzyme therapy for Fabry disease. Four companies are presently engaged in the development of enzyme therapy for Pompe disease. Genzyme/Pharming/Synpac as conglomerate and Novazyme (Oklahoma City, USA) as newcomer on the lysosomal disease market. As of August 2001, Genzyme and Novazyme have merged.

Back in 1991 there was no company strong and experienced enough to take up the challenge of producing recombinant acid AGLU for the treatment of Pompe disease, but research continued. Two Chinese hamster ovary (CHO) cell lines expressing high levels of recombinant enzyme were produced in university centres using the same AGLU cDNA but different vector systems [11, 36]. Using our CHO cell line, we produced a sufficient amount of enzyme to deliver proof of the principle of enzyme therapy in a mouse model of Pompe disease [7]. Simultaneously, therapeutic effect was shown in Japanese quails with the disease using the other CHO cell line [22]. Meanwhile, a completely different technology emerged which involved the production of medicines in the milk of transgenic animals [18]. Genomic DNA constructs are typically used in this production process in contrast to cDNA constructs employed in CHO cells (Fig. 2). The acid *AGLU* gene is linked to the promoter region of the bovine α_{S1}-casein gene that promotes high level expression in epithelial cells of the mammary gland. The construct is introduced in the animals' genome by injection into the pronucleus of fertilised oocytes. Embryos are implanted in foster mothers and a line of transgenic animals is obtained by germline transmission. The enzyme is harvested from the milk. Both production systems require downstream processing. The recombinant human acid AGLU from either milk or CHO cell media needs to undergo several rounds of purification before it can be administered intravenously to patients. The end products from CHO cells and milk are very similar in molecular mass (110 kD), and kinetic properties [7, 11, 36]. The carbohydrate composition may vary slightly depending on the enzyme source.

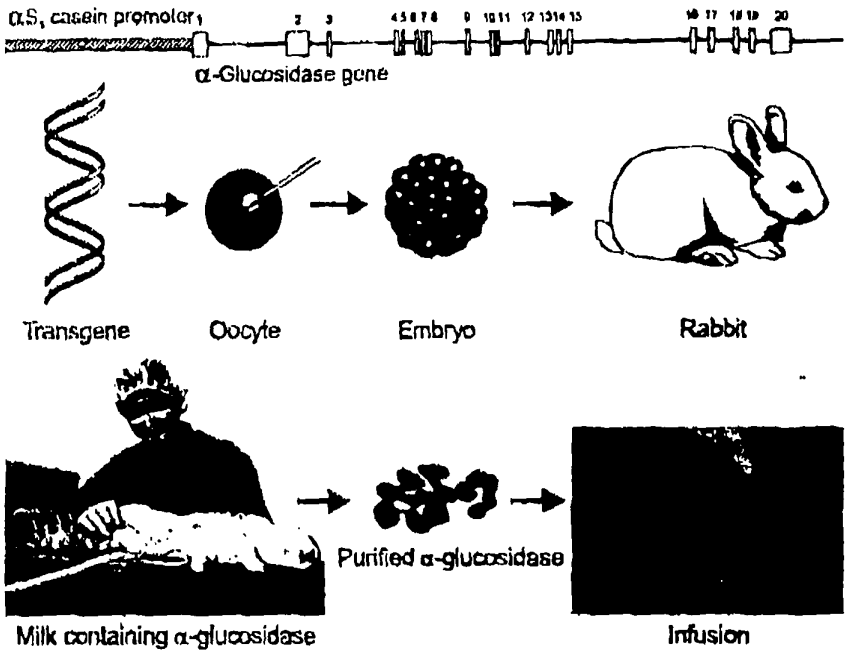

Fig. 2. Recombinant human AGLU from rabbit milk. The human acid *AGLU* gene is fused to 6.3 kb of the bovine α_{S1}-casein promoter and as such injected in the pronucleus of fertilised rabbit oocytes to generate transgenic founders. A transgenic line is obtained by breeding. Females produce recombinant human enzyme in the mammary gland during lactation and secrete the product in their milk. The enzyme is extracted from the milk in several purification steps and administered intravenously to the patients

Recombinant acid AGLU from CHO cells and mouse milk, both produced in our own laboratory, are equally efficiently taken up by cultured fibroblasts of patients with Pompe disease via the M6P receptor. However, the same enzyme from rabbit milk is taken up less efficiently by cultured fibroblasts. Interestingly, the uptake of the two enzymes by the target tissues of mice is not consistently different (unpublished results). More extensive studies are needed to verify these initial findings.

Clinical studies

Recombinant human acid AGLU from both sources were finally tested in the clinic (Table 1). Dr Chen of Duke University, North Carolina, USA reported at the SIIS symposium in Fulda the results of his study with CHO enzyme. We have summarised below the design and outcome of our study in which the enzyme from rabbit milk was used [31].

Study design and outcome

The aim of the study was to test safety and efficacy of recombinant human AGLU from rabbit milk in patients with the severe infantile form of Pompe disease. For inclusion, patients had to have the combination of generalised muscle weakness, cardiomegaly, acid AGLU deficiency and glycogen storage in skeletal muscle. Patients older than 10 months and those who were dependent on artificial ventilation were excluded. Four patients were included; two with an advanced stage of disease (7 and 8 months old), and two younger patients who were in significantly better condition at inclusion (2.5 and 3 months old). The two older patients were practically immobile at the time of inclusion. They required supplemental oxygen and had signs of cardiac instability. One of them became respirator dependent directly after inclusion before the start of treatment. The other patient became ventilator dependent after 10 weeks of treatment during a bout of pneumonia. One of the younger patients had signs of cardiac decompensation and respiratory distress at birth and was fed by nasogastric tube. The fourth patient was diagnosed at birth when he showed cardiomegaly on a chest X-ray film that was taken for disease unrelated indication. Both younger patients manifested axial hypotonia, head lag and slipping through.

The recombinant human AGLU from rabbit milk was administered intravenously in a weekly dose of 15–20 mg/kg (at start of treatment) to 40 mg/kg (at present) and is generally well tolerated. Transient reactions are seen sometimes during infusion, such as fever, malaise, erythematous rash, sweating, flushing and tachycardia. All are manageable without medication. After 12 weeks of treatment with the low dose, the acid AGLU activity in muscle had increased from 1%–2% of normal (before treatment) to 12%–28%, the levels typically measured in late-onset Pompe disease. During the 12 following weeks with high dose, the activity increased to normal levels. After 36 weeks of treatment, we observed improvement in muscle morphology in the younger patient who was in the best condition at the start of treatment. Muscle tissue sections stained less intense for glycogen (PAS staining) and muscle fibres appeared less damaged. Similarly clear

changes were at that time not yet evident in muscle biopsies of the other three patients.

Cardiac changes were monitored by ultrasound. The left ventricular mass index of all patients, exceeding approximately three times normal at inclusion, decreased after start of treatment (up to 25% of baseline for one of the patients). Signs of cardiac instability disappeared in all cases.

All patients gained strength over the first 36 weeks of treatment. They learned to play with toys. The two younger patients perform better than the two older ones. One of the younger patients learned to lift her legs from the surface and touch her feet whilst playing. At present she can sit independently. The other, with the best condition at start of treatment, has learned to sit and crawl at 9.5 months of age. At 12 months he could creep and stand with support of one arm and he could walk at 16 months. Importantly, all patients have well passed the age of 1 year which is more than the average life expectancy of patients with infantile Pompe disease. All four patients receive continuous treatment to evaluate the long-term effect of enzyme replacement therapy on motor and mental development and overall quality of life.

Results of two trials

Comparing the studies with recombinant human AGLU from rabbit milk and CHO cells, there are differences and similarities in outcome. One patient in each study responds very well. The patient treated with rabbit milk enzyme had a characteristic cardiomegaly at birth. The patient treated with CHO enzyme had a normal baseline cardiac evaluation with a left ventricular mass at two standard deviations above the norm (close to the P98) when treatment was started at 3 months after birth. Obviously, we are confronted with clinical diversity preventing in part the comparison of data. Further, dose level and infusion frequencies were different in the two trials. A second patient in the study with rabbit milk enzyme responds well in that she has acquired the ability to roll over and sit and has remained ventilator independent over the first 72 weeks. The patient is homozygote for the deltaT525 mutation and does not produce endogenous acid AGLU. Her progress demonstrates that a cross-reactive immunological material (CRIM) negative status is not a priori inhibitory for successful treatment. In contrast, the two CRIM negative patients in the trial with CHO enzyme were said to respond initially well to the treatment, but their condition declined when high antibody titres developed against the recombinant enzyme. The other patients in both trials are CRIM positive to some extent. The difference in antibody response is multi-interpretable. First of all, the enzyme preparations used are probably not identical with respect to precise molecular structure, notably the carbohydrate composition. Second, the two preparations may contain various types and degrees of impurities and are formulated differently. This may affect their immunogenicity. Moreover, the dosing regimen was different at the two test sites. All these factors, separate or together, may explain why CRIM negative patients respond differently to the two enzyme preparations.

Prospects and challenges

The positive effects of enzyme therapy for Pompe disease are too strong to deny. For the benefit of patients, enzyme therapy ought to be brought to the market. It requires a Phase III trial to deliver final proof of therapeutic effect. The current pilot studies with rabbit milk and CHO enzyme indicate that quick and convincing results can be obtained by extension of studies in patients with the infantile form of Pompe disease; but, at the same time, it seems inevitable that not all included patients will respond equally well. The problem can in part be managed by careful definition of inclusion and exclusion criteria and proper dosing. A second challenge is to prove efficacy of enzyme therapy in late onset Pompe disease. The milder affected patients live longer and are continuously confronted with loss of quality of life. It is essential for them to implement therapy at the earliest possible moment in order to prevent irreversible damage of muscle function. In theory, it is easier to correct the enzyme deficiency in late onset than in early onset disease because the residual AGLU activity is significantly higher in the former than in the latter condition. On the other hand, it has to be awaited whether adult muscle tissue is equally accessible for the enzyme and equally reparable as growing muscle of infants.

As it stands, the prospects of enzyme therapy for Pompe disease are good, but hurdles still need to be overcome. In April 2000, Genzyme-Pharming LLC announced the discontinued development of enzyme replacement therapy with recombinant human AGLU from rabbit milk. The companies stated that they believed production in CHO cells to be quicker. A new study with enzyme from CHO cells was started in May 2001. All together, that is more than 35 years after the first trials were undertaken. Patients, investigators and companies are eagerly awaiting the outcome.

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